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I also certify that the application is now proceeding in the name as identified herein.

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Signed Stephen Hordley

Dated 18 March 2004

GB 0225245.0

By virtue of a direction given under Section 30 of the Patents Act 1977, the application is proceeding in the name of

PROTEOME SCIENCES PLC,
Coveham House,
Downside Bridge Road,
COBHAM,
Surrey, KT11 3EP,
United Kingdom

Incorporated in the United Kingdom,

[ADP No. 07671670001]

Patents Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

310CT02 E759724-1 D01049
P01/7700 0.00-0225245.0

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Cardiff Road
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1. Your reference

ELEC,022-UK

2. Patent application number
(The Patent Office will fill in this part)

0225245.0

30 OCT 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

UNIVERSITE DE GENEVE
Rue General-Dufour 24
Case Postale
CH-1211 Geneva
Switzerland

SECTION 30 (1977 ACT) APPLICATION FILED 4/6/03

Patents ADP number *(if you know it)*

115365502

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

DIAGNOSTIC METHOD FOR
TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

LUCAS & CO
135 Westhall Road
Warlingham
Surrey CR6 9HJ

Patents ADP number *(if you know it)*

05815709001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

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a) any applicant named in part 3 is not an inventor, or
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Request for preliminary examination and search *(Patents Form 9/77)*	
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11. I/We request the grant of a patent on the basis of this application.

Signature Paul Cole Date 30 OCT 02

12. Name and daytime telephone number of person to contact in the United Kingdom PAUL GILBERT COLE 01883 626211

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NP9 1RH

1. Your reference

ELEC,022-UK

2. Patent application number
(if you know it)

02 25245.0

3. Full name of the or of each applicant

Université de Genève

4. Title of the invention

Diagnostic method for Transmissible Spongiform Encephalopathies

5. State how the applicant *(s)* derived the right from the inventor *(s)* to be granted a patent

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3

7. I/We believe that the person *(s)* named over the page *(and on any extra copies of this form)* is/are the inventor *(s)* of the invention which the above patent application relates to.

Signature: Brian Lucas

Date: 26/Nov/2002

8. Name and daytime telephone number of person to contact in the United Kingdom

Brian Lucas 01883 626211

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Enter the full names, addresses and postcodes of the inventors in the boxes and underline the surnames

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Chemin de la Savonniere 27
Collonge-Bellerive
CH-1245 Geneva
Switzerland

8520660001

Patents ADP number *(if you know it)*:

SANCHEZ, Jean-Charles
Chemin Frank-Thomas 42
CH-1208 Geneva
Switzerland

7690910001

Patents ADP number *(if you know it)*:

GUILLAUME, Elisabeth
8A Rue du Capitaine Charles
Dupraz
F-74100 Annemasse
France

8231276aa

Patents ADP number *(if you know it)*:

Reminder

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DIAGNOSTIC METHOD FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

BACKGROUND OF THE INVENTION

Field of the invention

This invention relates to a diagnostic method for a transmissible spongiform encephalopathy (TSE).

Description of the related art

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases of the central nervous system. They can be transmitted, inherited or occur sporadically and are observed in animals, e.g. as bovine spongiform encephalopathy (BSE) in cattle or scrapie in sheep, as well as in humans as Creutzfeldt-Jakob disease (CJD), Gerstman Sträussler Scheinker syndrome, Fatal Familial Insomnia or Kuru. They have a long incubation period, leading to ataxia, dementia, psychiatric disturbances and death. Neuropathological changes include vacuolar degeneration of brain tissue, astrogliosis and amyloid plaque formation. The diseases are difficult to diagnose pre-mortem.

The cerebrospinal fluid (CSF) of CJD patients displays two additional polypeptides (known as 14-3-3 polypeptides) by two-dimensional polyacrylamide gel electrophoresis [Harrington, M.G. New England Journal of Medicine *315*, 279 (1986), Hsich, G., Kenney, K., Gibbs, C.J., Lee, K.H. & Harrington, M. B. New England Journal of Medicine *335*, 924 (1996).] The function of these 14-3-3 polypeptides remains unclear in TSE. They can be used in a pre-mortem test for CJD diagnostic evaluation, but have low specificity.

Monoclonal antibodies to the abnormal form of prion protein (which is associated with CJD) are available and can be used in an enzyme-linked immunoassay, as described in PCT Specifications WO 98/23962 and 98/32710 and Schmerr, M.J., the Beckman Coulter Pace Setter Newsletter 3(2), 1-4 (June 1999), but these procedures have not yet been fully developed.

PCT/EP 01/02894 relates to a diagnostic assay for TSEs in which the concentration of heart or brain fatty acid binding protein (H-FABP or B-FABP) is determined in a sample of body fluid.

PCT/EP 02/????) describes a method for aiding TSE diagnosis, which comprises determining a test amount of a polypeptide marker, which is differentially present in samples of TSE subject and a subject who does not have TSE. The marker may be determined in body fluids using mass spectrometry, and preferably laser desorption mass spectrometry

US-A-6225047 describes the use of retentate chromatography to generate difference maps, and in particular a method of identifying analytes that are differentially present between two samples. One specific method described therein is laser desorption mass spectrometry.

WO 01/25791 describes a method for aiding a prostate cancer diagnosis, which comprises determining a test amount of a polypeptide marker, which is differentially present in samples of a prostate cancer patient and a subject who does not have prostate cancer. The marker may be determined using mass spectrometry, and preferably laser desorption mass spectrometry.

Development of new non-invasive TSE markers for body fluids (in particular, CJD and BSE markers in blood) and new methods of determining the markers would help

clinicians to establish early diagnosis. This problem has now been solved by the present invention.

SUMMARY OF THE INVENTION

The present invention provides a method of diagnosis of a transmissible spongiform encephalopathy (TSE) or the possibility thereof in a subject suspected of suffering from the TSE, which comprises subjecting a sample of body fluid taken from the subject to mass spectrometry, thereby to determine a test amount of a polypeptide in the sample, wherein the polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-TSE-infected subjects, and has a molecular weight in the range of from 1000 to 100000; and determining whether the test amount is consistent with a diagnosis of TSE.

The invention also provides use of a polypeptide which is differentially contained in a body fluid of TSE-infected subjects and non-infected subjects, the polypeptide having a molecular weight in the range of from 1000 to 100000 and being determinable by mass spectrometry, for diagnostic, prognostic and therapeutic applications.

The invention further provides a kit for use in diagnosis of TSE, comprising a probe or a protein chip array for receiving a sample of body fluid, and for placement in a mass spectrometer, thereby to determine a test amount of a polypeptide in the sample, wherein the polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-TSE-infected subjects, and has a molecular weight in the range of from 1000 to 100000.

In embodiments of the invention, the molecular weight may, for example, be from 1000 to less than 3500, from 3500 to 30000, or from above 30000 to 100000.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A to 1F are spectral views of plasma from normal and BSE-infected samples using laser desorption/ionization mass spectrometry, highlighting protein peaks at about 1010, 1100, 1125, 1365, 3645, 4030, 3890, 5820, 7520, 7630, 7980, 9950, 10250, 11600, 11800, 15000, 15200, 15400, 15600, 15900, 30000, 31000 and 31800 Da in plasma samples.

DESCRIPTION OF PREFERRED EMBODIMENTS

The invention provides a method of diagnosis of a transmissible spongiform encephalopathy (TSE) or the possibility thereof in a subject suspected of suffering from the TSE. A sample of body fluid taken from the subject is subjected to mass spectrometry, to determine the presence or absence in the sample of a polypeptide marker, which is differentially contained in the body fluid of TSE-infected subjects and non-infected subjects. The polypeptide marker has a molecular weight in the range of from 1000 to 100000, preferably from 1000 to 35000, and the presence, absence, under-expression or over-expression of the marker is indicative of TSE.

The method is applicable to all types of TSE, and to any human or animal suffering or suspected of suffering therefrom. The method is especially applicable to the diagnosis of CJD, especially new variant CJD, in human patients, and to BSE in ruminant animals such as cattle, and to BSE-like diseases in other animals, such as scrapie in sheep.

The term polypeptide includes proteins and protein fragments, as well as peptides modified by the addition of non-peptide residues, e.g. carbohydrates, phosphates, sulfates or any other post-translational modification.

The sample may be adsorbed on a probe under conditions which allow binding between the polypeptide and adsorbent material on the probe or the protein chip array. The adsorbent material preferably comprises a metal chelating group complexed with a metal ion, and a preferred metal is copper. Prior to detecting the polypeptide,

unbound or weakly bound materials on the probe or protein chip array may be removed with a washing solution, thereby enriching the polypeptide in the sample. The sample is preferably adsorbed on a probe or protein chip array having an immobilised metal affinity capture (IMAC) surface capable of binding the polypeptide. The sample may be also adsorbed on a probe having hydrophobic, strong anionic or weak cationic exchange surfaces under conditions which allow binding of the polypeptides. The probe may consist of a strip having several adsorbent wells, and be inserted into the spectrometer, then movable therein so that each well is in turn struck by the ionizing means (e.g. laser) to give a spectrometer reading. The polypeptide is preferably determined by surface-enhanced laser desorption/ionisation (SELDI) and time of flight mass spectrometry (TOF-MS).

In principle, any body fluid or tissue can be used to provide a sample for diagnosis, but preferably the body fluid is cerebrospinal fluid (CSF), plasma, serum, blood, urine, saliva or tears.

In one embodiment of the invention, the TSE is bovine spongiform encephalopathy (BSE). In this case, the polypeptide preferably has a molecular weight of about 1010, 1100, 1125, 1365, 3645, 4030, 3890, 5820, 7520, 7630, 7980, 9950, 10250, 11600, 11800, 15000, 15200, 15400, 15600, 15900, 30000, 31000 and 31800 Da and the presence, absence, over-expression or under-expression of the polypeptide is indicative of BSE.

In another embodiment of the invention, the TSE is CJD.

In a further embodiment of the invention, the TSE is scrapie.

Measurement of the molecular weight of the polypeptide or polypeptides is effected in the mass spectrometer. All molecular weights herein are measured in Da. The molecular weights quoted above can be measured with an accuracy of better than 1%, generally 0.5 to 1%, and preferably to within about 0.1%. The term "about" in connection with molecular weights in this specification therefore means within a

variation of about 1%, preferably 0.5%, and more preferably within about 0.1%, above or below the quoted value.

The invention also relates to the use of a polypeptide which is differentially contained in a body fluid of TSE-infected subjects and non-infected subjects, the polypeptide having a molecular weight in the range of from 1000 to 100000 and being determinable by mass spectrometry, for diagnostic, prognostic and therapeutic applications. This may involve the preparation and/or use of a material, which recognizes, binds to or has some affinity to the above-mentioned polypeptide. Examples of such materials are antibodies and antibody chips. The term "antibody" as used herein includes polyclonal antiserum, monoclonal antibodies, fragments of antibodies such as Fab, and genetically engineered antibodies. The antibodies may be chimeric or of a single species. The above reference to "prognostic" applications includes making a determination of the likely course of a TSE by, for example, measuring the amount of the above-mentioned polypeptide in a sample of body fluid. The above reference to "therapeutic" applications includes, for example, preparing materials which recognize, bind to or have affinity to the above-mentioned polypeptides, and using such materials in therapy. The materials may in this case be modified, for example by combining an antibody with a drug, thereby to target the drug to a specific region of the animal to be treated.

The methodology of this invention can be applied to the diagnosis of any TSE. Body fluid samples are prepared from infected and non-infected subjects. The samples are applied to a probe or array having a surface treated with a variety of adsorbent media, for differential retention of peptides in the sample, optionally using washing liquids to remove unbound or weakly bound materials. If appropriate, energy-absorbing material can also be applied. The probe or array is then inserted into a mass spectrometer, and readings are taken for the various sample/adsorbent combinations using a variety of spectrometer settings. Comparison of the infected and non-infected samples under a given set of conditions reveals one or more polypeptides, which are differentially expressed in the infected and non-infected samples. The presence or absence of these polypeptides can then be used in the testing of a fluid sample from a

subject under the same conditions (adsorbent, spectrometer settings etc.) to determine whether or not the subject is infected.

References herein to "presence or absence" of a polypeptide should be understood to mean simply that there is a significant difference in the amount of a polypeptide which is detected in the infected and non-infected sample. Thus, the "absence" of a polypeptide in a test sample may include the possibility that the polypeptide is actually present, but in a significantly lower amount than in a comparative test sample. According to the invention, a diagnosis can be made on the basis of the presence or absence of a polypeptide, and this includes the presence of a polypeptide in a significantly lower or significantly higher amount with reference to a comparative test sample.

The following Examples illustrate the invention.

EXAMPLE 1

The objective of the present study was to detect specific polypeptides in body fluids (cerebrospinal fluid, plasma and others) of BSE affected cattle. Samples were analysed by the Surface Enhanced Laser Desorption Ionization (SELDI) Mass Spectroscopy (MS) technology. This technology encompasses micro-scale affinity capture of proteins by using different types of retentate chromatography and then analysis by time of flight mass spectrometry. Different maps are thus generated each corresponding to a typical protein profiling of given samples that were analysed with a Ciphergen Biosystem PBS II mass spectrometer (Freemont, CA, USA). Differential expressed peaks were identified when comparing spectra generated in a group of plasma samples from BSE-affected cattle with a group of healthy cattle using protein chip arrays.

The SELDI analysis was performed using 2µl of crude bovine plasma samples in order to detect specific polypeptides with metal affinity. An immobilized copper affinity array (IMAC-Cu^{++}) was employed in this approach to capture proteins with affinity for copper to select for a specific subset of proteins from the samples. It will

be appreciated that other protein chip arrays and immobilized metal chip arrays may be substituted for the IMAC-Cu^{++} affinity array. Captured proteins were directly detected using the PBSII Protein Chip Array reader (Ciphergen Biosystems, Freemont, CA, USA).

The following protocol was used for the processing and analysis of ProteinChip arrays using Chromatographic TED-Cu(II) adsorbent array. TED is a (tris(carboxymethyl)ethylenediamine-Cu) adsorbent coated on a silicon oxide-coated stainless steel substrate.

- The surface was first loaded with 10 µl of 100 mM copper sulfate to each spot and incubated for 15 minutes in a wet chamber.
- The chip was thereafter washed by two quick rinses with deionized water for about 10 seconds to remove the excess unbound copper.
- Before loading the samples, the I-MAC 3 array was equilibrated once with 5 µl of PBS NaCl 0.5 M for 5 minutes.
- After removing the equilibration buffer, 3 µl of the same buffer were added before applying 2 µl of plasma. The chip was incubated for 20 minutes in a wet chamber.
- The samples were thereafter removed and the surface was washed three times with the equilibration buffer (5 minutes each).
- Two quick final rinses with water were performed.
- The surface was allowed to air dry, followed by the addition of 0.5 µl of saturated sinapinic acid (SPA, Ciphergen Biosystem) prepared in 50% acetonitrile, 0.5% trifluoroacetic acid.
- The chip was air dried again before analysis of the retained protein on each spot with laser desorption/ionization time-of-flight mass spectrometry.
- The protein chip array was inserted into the instrument and analysed once the appropriate detector sensitivity and laser energy have been established to automate the data collection.
- The obtained spectra were analysed with the Biomark Wizard software (Ciphergen Biosystems, Freemont, CA, USA) running on a Dell Dimension 4100 PC. It generates consistent peak sets across multiple spectra.

Figures 1A-1F show the results of a comparative study, which has been undertaken between plasma from BSE diagnosed cattle and normal plasma, using the IMAC3 protein chip array prepared as described above. In this study, we found that 23 peaks were significantly differentially expressed in plasma from BSE affected cattle. Their molecular weights are respectively about 1010, 1100, 1125, 1365, 3645, 4030, 3890, 5820, 7520, 7630, 7980, 9950, 10250, 11600, 11800, 15000, 15200, 15400, 15600, 15900, 30000, 31000 and 31800 Da (mass accuracy is around 0.1%). Figure 1 shows two spectral views, respectively of the normal and BSE samples, from 0 to 100,000 Da. More specifically, as indicated by the vertical arrows, Figure 1A shows the peaks at about 1010, 1100, 1125 and 1365. Figure 1B shows the peaks at about 3645 and 4030. Figure 1C shows the peaks at about 3890, 5820, 7520, 7630 and 7980. Figure 1D shows the peaks at about 9950, 10250, 11600 and 11800. Figure 1E shows the peaks at about 15000, 15200, 15400, 15600 and 15900. Figure 1F shows the peaks at about 30000, 31000 and 31800.

Spectra P1 to P20 (Figures 1A-1B) correspond to a batch of samples from UK, and spectra 1 to 20 (Figures 1C-1F) correspond to a batch of samples from US. The status of the cattle providing the samples is indicated below in Tables 1 and 2, where negative means not affected by BSE and positive means BSE affected cattle.

Table 1

#	Type	Status
P1	Plasma	Negative
P2	Plasma	Negative
P3	Plasma	Positive
P4	Plasma	Negative
P5	Plasma	Positive
P6	Plasma	Positive
P7	Plasma	Negative
P8	Plasma	Negative
P9	Plasma	Positive
P10	Plasma	Negative
P11	Plasma	Positive
P12	Plasma	Positive
P13	Plasma	Positive
P14	Plasma	Positive
P15	Plasma	Negative
P16	Plasma	Negative

P17	Plasma	Negative
P18	Plasma	Positive
P19	Plasma	Positive
P20	Plasma	Negative

Table 2

#	Type	Status
1	Plasma	Positive
2	Plasma	Positive
3	Plasma	Positive
4	Plasma	Positive
5	Plasma	Positive
6	Plasma	Positive
7	Plasma	Positive
8	Plasma	Positive
9	Plasma	Positive
10	Plasma	Positive
11	Plasma	Positive
12	Plasma	Negative
13	Plasma	Negative
14	Plasma	Negative
15	Plasma	Negative
16	Plasma	Negative
17	Plasma	Negative
18	Plasma	Negative
19	Plasma	Negative
20	Plasma	Negative

These data demonstrate that the peaks of about 1010, 1100, 1125, 1365, 3645, 4030, 3890, 5820, 7520, 7630, 7980, 9950, 10250, 11600, 11800, 15000, 15200, 15400, 15600, 15900, 30000, 31000 and 31800 Da can be used to diagnose BSE in plasma samples.

* * * * *

Each of the above cited publications is herein incorporated by reference to the extent to which it is relied on herein.

CLAIMS

1. A method of diagnosis of a transmissible spongiform encephalopathy (TSE) or the possibility thereof in a subject suspected of suffering from the TSE, which comprises subjecting a sample of body fluid taken from the subject to mass spectrometry, thereby to determine a test amount of a polypeptide in the sample, wherein the polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-TSE-infected subjects, and has a molecular weight in the range of from 1000 to 100000; and determining whether the test amount is consistent with a diagnosis of TSE.

2. A method according to Claim 1, in which the polypeptide is present in the body fluid of TSE-infected subjects and not present in the body fluid of non-TSE-infected subjects, whereby the presence of the polypeptide in a body fluid sample is indicative of TSE.

3. A method according to Claim 1, in which the polypeptide is not present in the body fluid of TSE-infected subjects and present in the body fluid of non-TSE-infected subjects, whereby the non-presence of the polypeptide in a body fluid sample is indicative of TSE.

4. A method according to any of Claims 1 to 3, in which the mass spectrometry is laser desorption/ionization mass spectrometry.

5. A method according to any of Claims 1 to 4, in which the sample is adsorbed on a probe or on a protein chip array having an immobilised metal affinity capture (IMAC), hydrophobic, strong anionic or weak cationic exchange surface capable of binding the polypeptide.

6. A method according to any of Claims 1 to 5, in which the polypeptide is determined by surface-enhanced laser desorption/ionisation (SELDI) and time of flight mass spectrometry (TOF-MS).

7. A method according to any of Claims 1 to 6, in which the body fluid is cerebrospinal fluid, plasma, serum, blood, tears, urine or saliva.

8. A method according to any of Claims 1 to 7, in which a plurality of peptides is determined in the sample.

9. A method according to any of Claims 1 to 8, in which the TSE is Bovine Spongiform Encephalopathy (BSE).

10. A method according to Claim 9, in which one or more polypeptides having a respective molecular weight of about 1010, 1100, 1125, 1365, 3645, 4030, 3890, 5820, 7520, 7630, 7980, 9950, 10250, 11600, 11800, 15000, 15200, 15400, 15600, 15900, 30000, 31000 and 31800 Da is determined, and the differential expression of one or more of such polypeptides is indicative of BSE.

11. A method according to any of Claims 1 to 8, in which the TSE is Creutzfeldt-Jackob Disease (CJD).

12. A method according to any of Claims 1 to 8, in which the TSE is scrapie.

13. Use of a polypeptide which is differentially contained in a body fluid of TSE-infected subjects and non-infected subjects, the polypeptide having a molecular weight in the range of from 1000 to 100000 and being determinable by mass spectrometry, for diagnostic, prognostic and therapeutic applications.

14. Use for diagnostic, prognostic and therapeutic applications of a material which recognizes, binds to or has affinity for a polypeptide which is differentially contained in a body fluid of TSE-infected subjects and non-infected subjects, the polypeptide having a molecular weight in the range of from 1000 to 100000 and being determinable by mass spectrometry.

15. Use according to Claim 14, in which the material is an antibody or antibody chip.

16. A kit for use in diagnosis of TSE, comprising a probe for receiving a sample of body fluid, and for placement in a mass spectrometer, thereby to determine a test amount of a polypeptide in the sample, wherein the polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-TSE-infected subjects, and has a molecular weight in the range of from 1000 to 100000.

17. A kit according to Claim 16, in which the probe contains an adsorbent for adsorption of the polypeptide.

18. A kit according to Claim 17, further comprising a washing solution for removal of unbound or weakly bound materials from the probe.

ABSTRACT

DIAGNOSTIC METHOD FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

Transmissible spongiform encephalopathy (TSE) is diagnosed in a subject by using mass spectrometry to observe a polypeptide in a sample of body fluid taken from the subject. The polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-infected subjects, and has a molecular weight in the range of from 1000 to 100000.

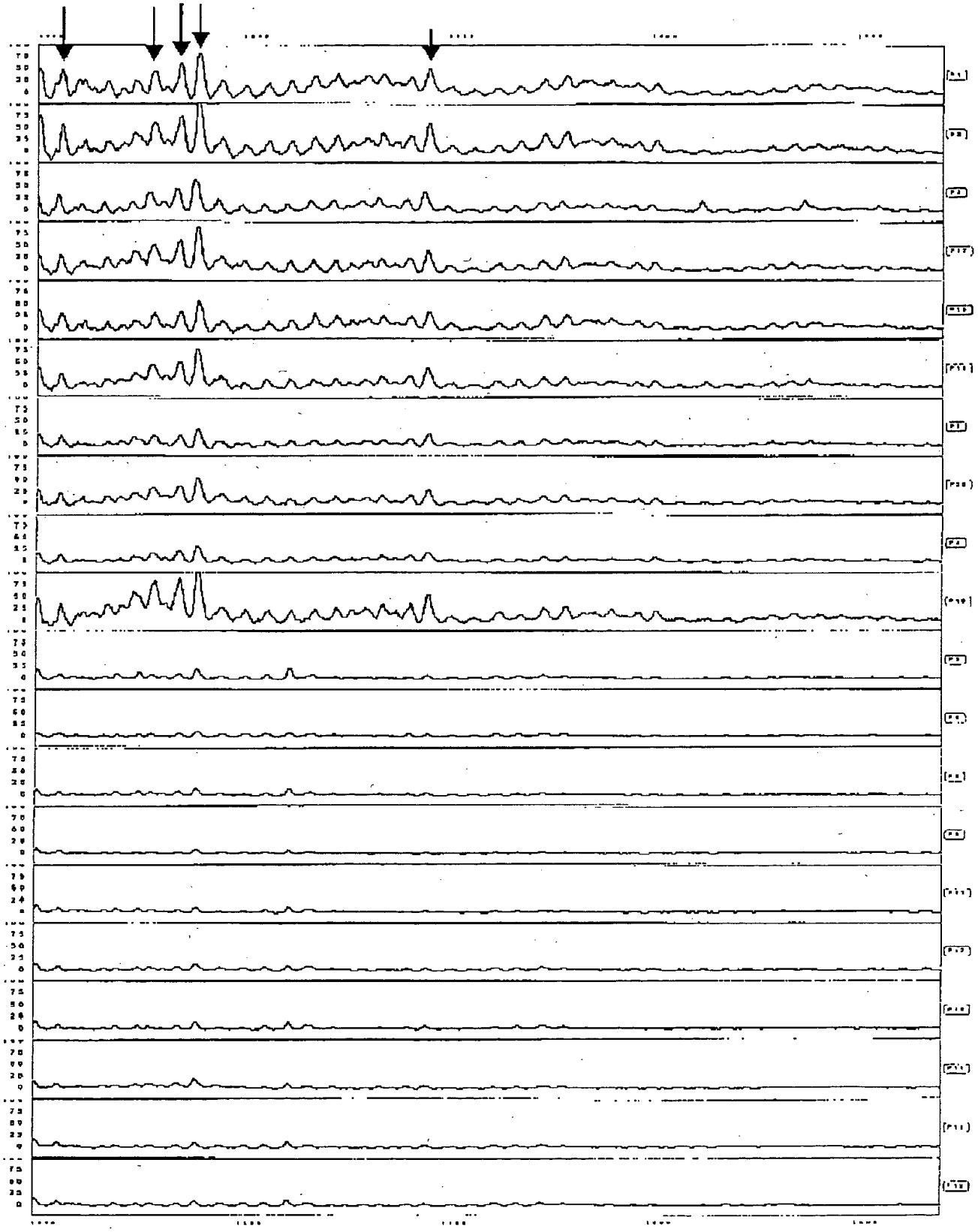

Figure 1.A

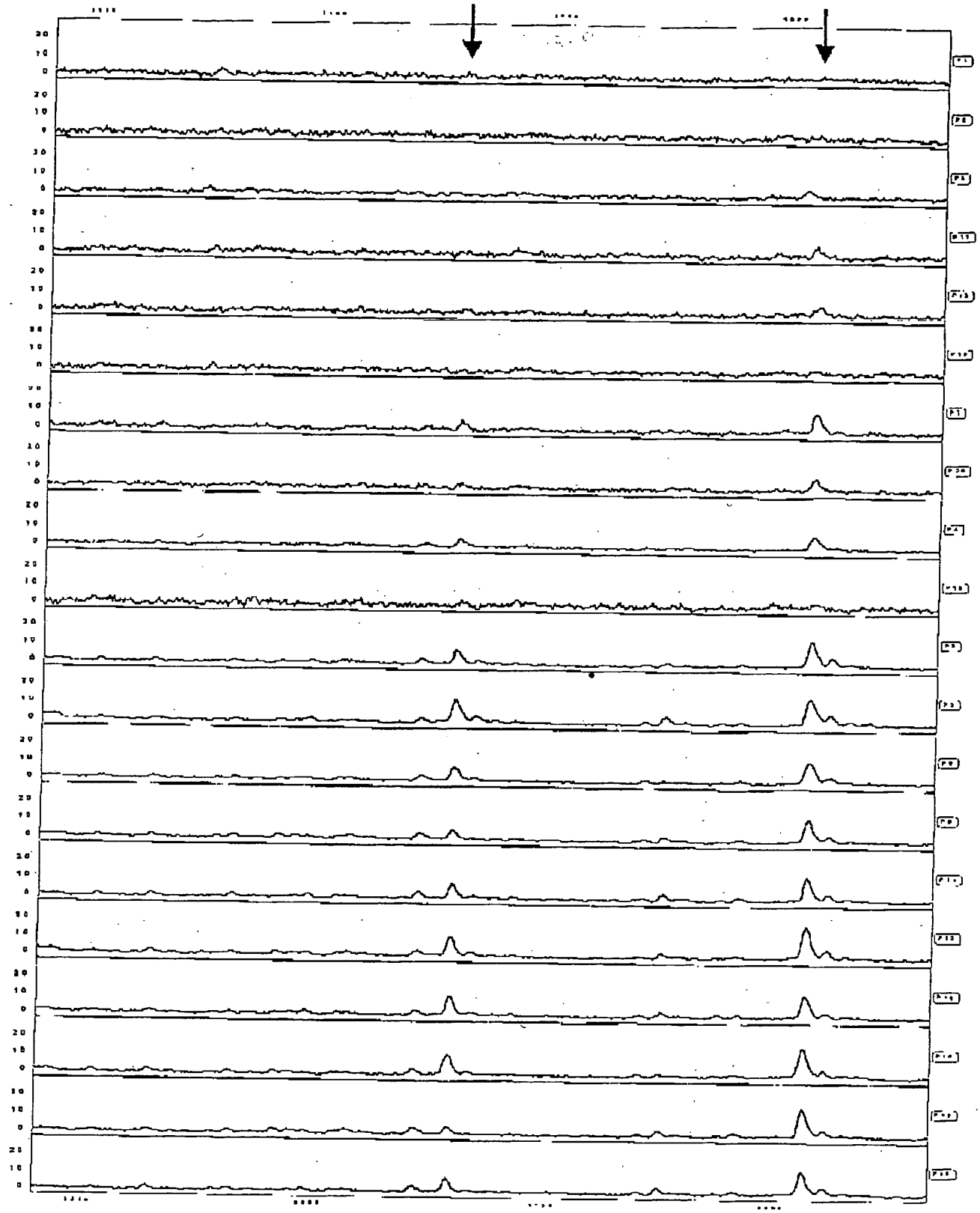

Figure 1.B

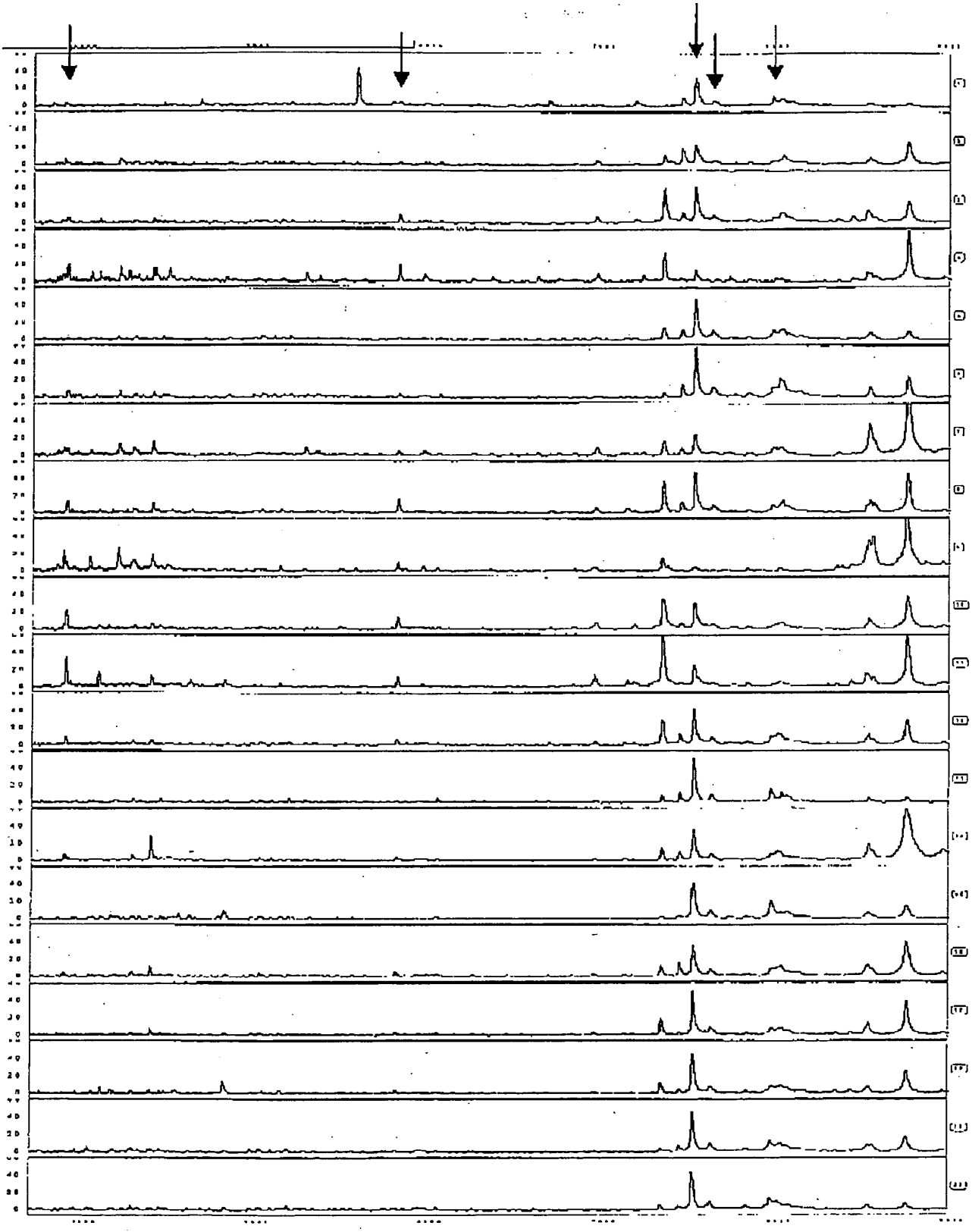

Figure I.C

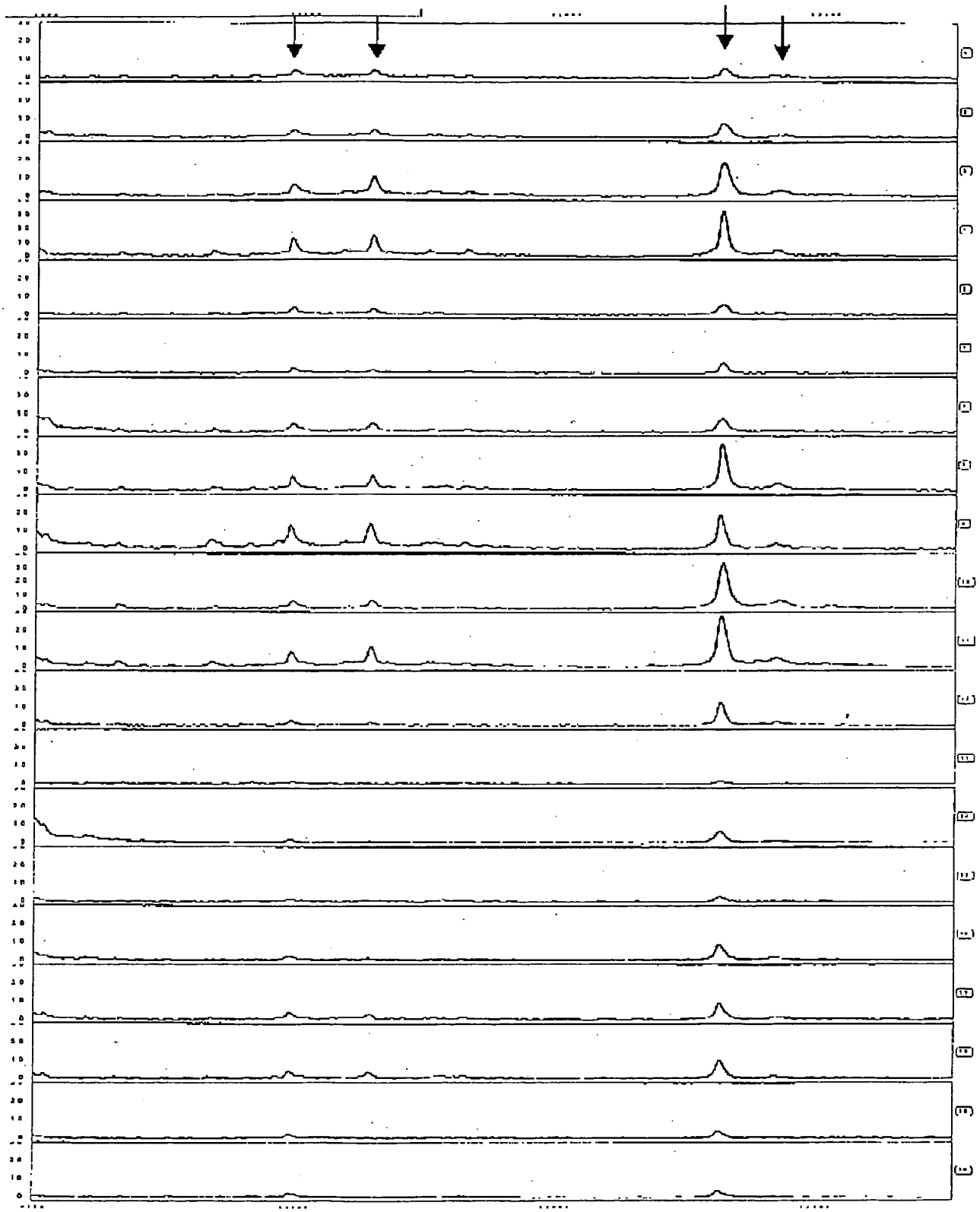

Figure 1.D

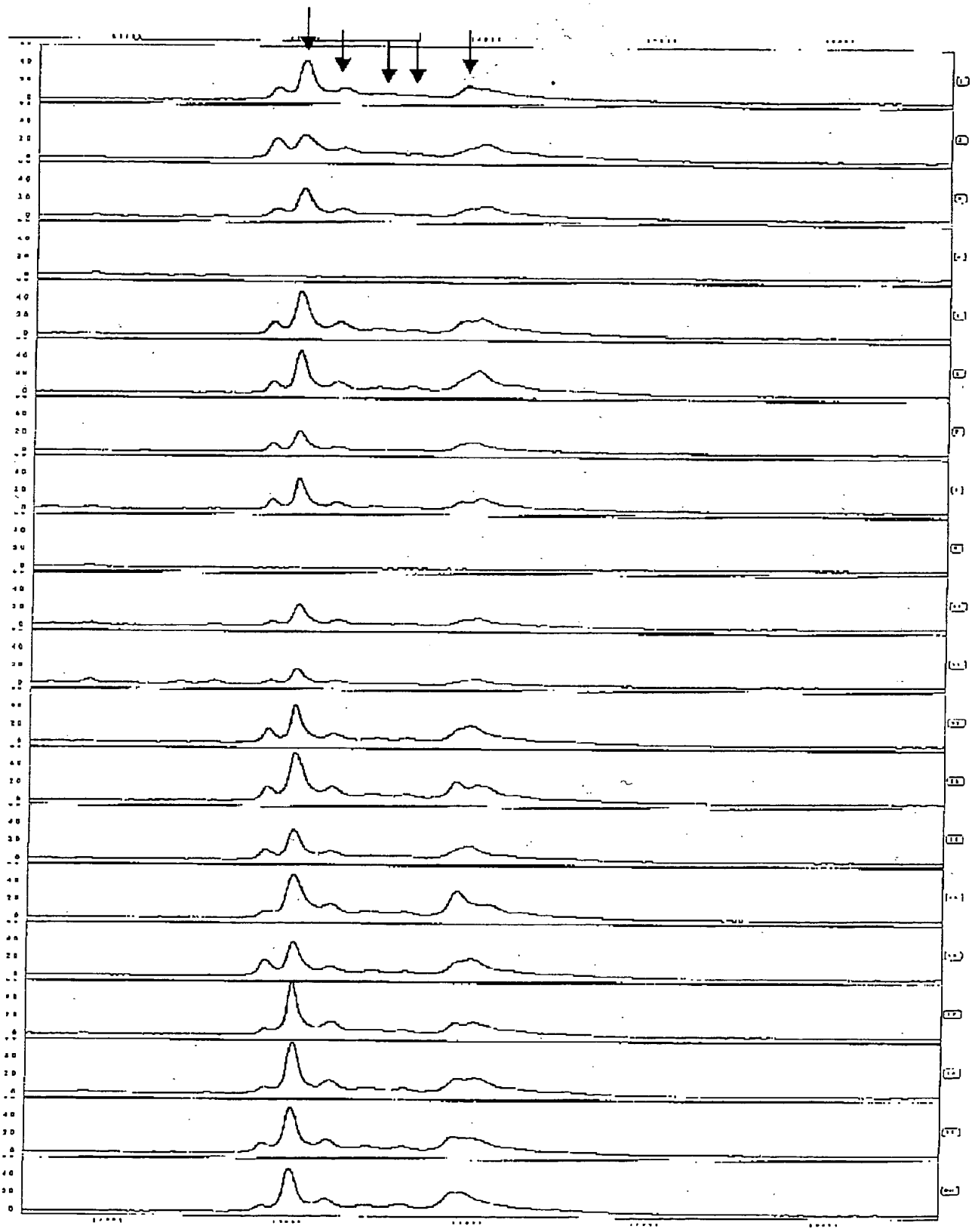

Figure 1.E

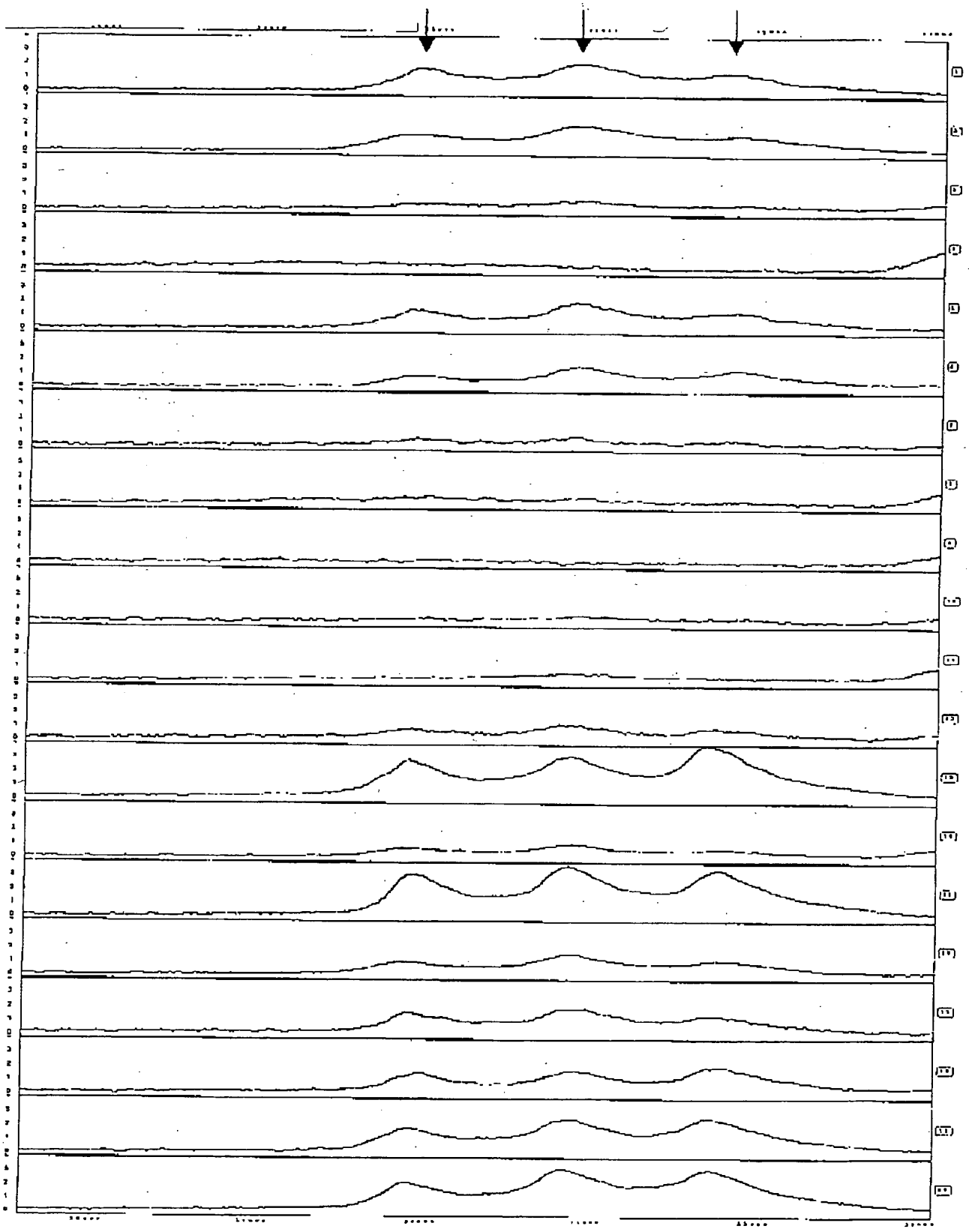

Figure 1.F